Comprehensive Characterization of LEDGF/p75 in an HIV-1 infected Patient Cohort

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BACKGROUND:

Lens epithelium derived growth factor interaction inhibitors (LEDGINs) form an emerging class of allosteric integrase inhibitors, targeting the cellular co-factor of integrase, i.e. LEDGF/p75. Only few data are available on LEDGF/p75 expression and its genetic variability in HIV infected patients. The present study evaluated whether genetic variation in the LEDGF/p75 gene and mRNA/protein expression levels influence HIV disease progression.

METHODS & RESULTS:

Patients: Samples were derived from a therapy-naive patient cohort from Ghent University Hospital and from long-term-non-progressor patients as kindly provided by the HIV Biobank (Spanish AIDS Research Network, RIS). Elite controllers are defined as therapy-naive long-term-non-progressors (LTNP) with undetectable viral load, LTNP viremic controllers have a viral load below 2000 copies/ml without therapy in 75% of the measurements, LTNP viremic non controllers are therapy-naive and harbor a viral load >2000 copies/ml. Normal progressors are non-LTNP with a CD4 decline less than 100 cells/µl*year and rapid progressors are non-LTNP patients with CD4 decline of more than 100 cells/µl*year.

Table 1: Patient characteristics

Cohort		Ghent Cohort N=187	RIS Cohort N=138	Overall N=325
Ethnicity, N (%)	African	34 (18,2)	0 (0)	34 (10,5)
	Caucasian	153 (81,2)	138 (100)	291 (89,5)
Disease progression N (%)	LTNP - Elite controller	6 (3,2)	42 (30,4)	48 (14,8)
	LTNP - Viremic controller	16 (8,6)	47 (34,1)	63 (19,4)
	LTNP - Non controller	17 (9,1)	49 (35,5)	66 (20,3)
	Normal progressor	113 (60,4)	0 (0,0)	113 (34,8)
	Rapid progressor	35 (18,7)	0 (0,0)	35 (10,8)

SNP detection: A genomic scan of the coding region (including intronic regions near the exon-intron boundary and 3'UTR) of LEDGF/p75 was performed with high resolution melting (HRM) curve analysis and Sanger sequencing to identify single nucleotide polymorphisms (SNPs). 24 SNPs were identified, of which 5 in the coding region, 17 in the non-coding regions and 3'UTR. In addition to these, two known tagSNPs were included. Only the SNPs that were correlated with disease progression, average viral load or CD4 slope are described (Fig. 1).

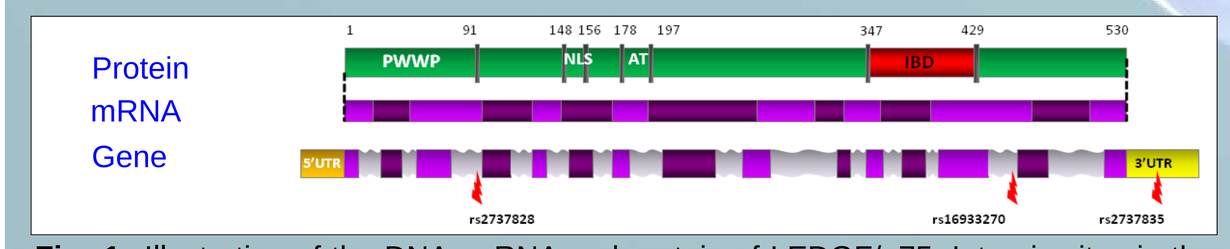


Fig. 1: Illustration of the DNA, mRNA and protein of LEDGF/p75. Intronic sites in the DNA are grey, and the positions of the SNPs that are discussed are marked.

LEDGF mRNA expression: mRNA expression levels were determined using RT-qPCR with validated reference genes for normalization. This revealed a stable expression of the mRNA in most patient samples (Fig. 2).

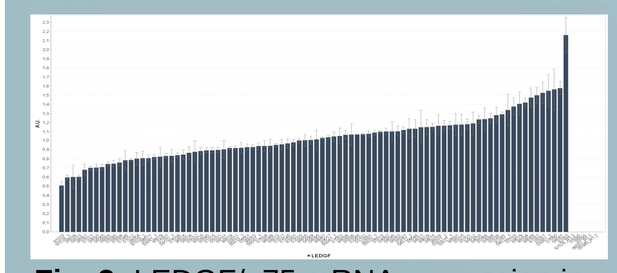


Fig. 2: LEDGF/p75 mRNA expression in all patients

Western Blotting: A high variability of LEDGF/p75 expression was observed using β-ACTIN as loading control. This variability was also observed in biological replicate samples and was independent of LEDGF/p75 mRNA levels (Fig. 3).

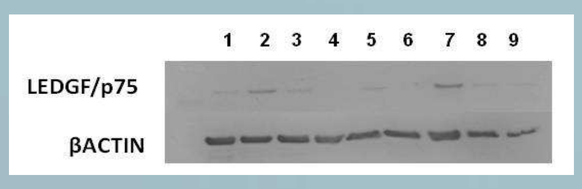
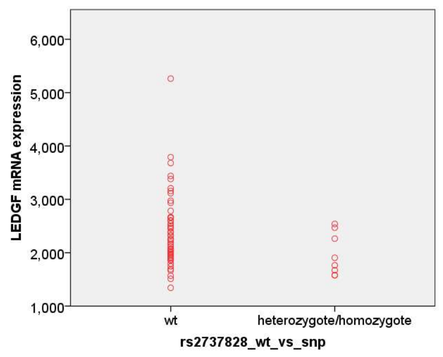


Fig. 3: Western blot on patient samples, 1,2,3-4,6,9: normal progressors, 1&2 and 8&9: biological replicates, 3: viremic controller, 5&7 elite controllers

Association of LEDGF/p75 SNPs with disease progression

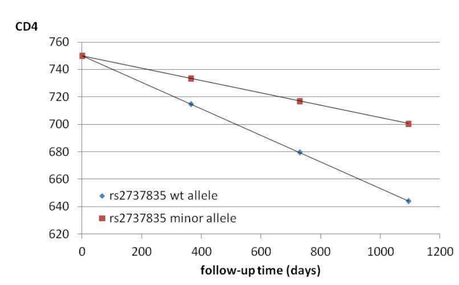
The SNPs in the coding region were low-abundant and did not correlate with disease progression nor with LEDGF/p75 expression. For most of the intronic and 3'UTR SNPs found, no correlation could be determined with either CD4 slope, viral load or LEDGF/p75 expression. However, for three SNPs, a possible association between SNP presence and HIV disease were found, i.e. rs2737828, rs2737835 and rs16933270.

rs2737828



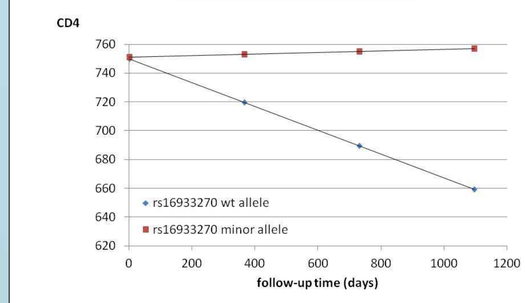
This intronic SNP was under-represented in Caucasian HIV patients (P<0.0001) compared to healthy Caucasians as published in HapMap. In addition, the presense of this SNP tends to correlate with lower LEDGF/p75 expression (P=0.053), but not with disease progression markers.

rs2737835



This SNP tends to be associated with slower CD4 decline in Caucasian non controllers (P=0.058).

rs16933270



This SNP, mainly present in Africans was correlated with CD4 decline (P= 0.020).

DISCUSSION:

This is the first report on SNP profiling in the entire coding region of the LEDGF/p75 gene in HIV infected patients. The results reveal that the coding region contains little variation. Certain rare variants of LEDGF/p75 intronic or 3'UTR region can be associated with HIV disease susceptibility or disease progression.

The variant rs2737835, present in Caucasian patients, tends to associate with slower CD4 decline. rs16933270, mainly present in African patients significantly correlated with CD4 decline. These date indicate that genetic variations in LEDGF/p75 can influence disease progression.

The finding that SNP rs2737828 was significantly underrepresented in Caucasian HIV patients in relation to the expected frequency according to HapMap, suggests that this SNP might influence disease susceptibility.

Interestingly, except for rs2737828, there was no correlation between the presence of these variants and differential expression at the mRNA and protein levels. The high variety of protein expression that is not correlated with LEDGF/p75 mRNA expression suggests that post transcriptional modifications are important for protein expression. This might have implications for the pharmacodynamic impact of LEDGIN in vivo.

CONCLUSIONS:

This study supports the hypothesis that genetic variation in the host factors LEDGF/p75 can influence HIV disease progression.

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